

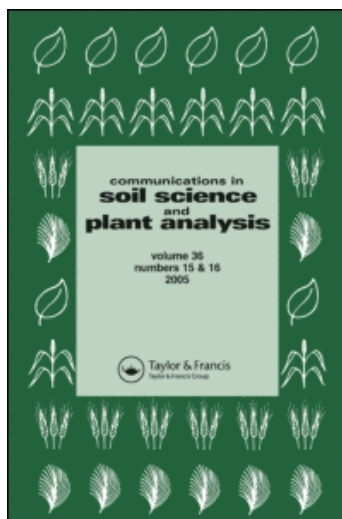
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### Manure Phosphorus Fractions: Development of Analytical Methods and Variation with Manure Types

Martha Mamo <sup>a</sup>; Charles Wortmann <sup>a</sup>; Corey Brubaker <sup>b</sup>

<sup>a</sup> Department of Agronomy and Horticulture, University of Nebraska, Lincoln, Nebraska, USA <sup>b</sup> USDA-NRCS, Lincoln, Nebraska, USA

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## **Manure Phosphorus Fractions: Development of Analytical Methods and Variation with Manure Types**

**Martha Mamo and Charles Wortmann**

Department of Agronomy and Horticulture, University of Nebraska,  
Lincoln, Nebraska, USA

**Corey Brubaker**

USDA-NRCS, Lincoln, Nebraska, USA

**Abstract:** Manure phosphorus (P) extraction and storage procedures were evaluated, and manure types were characterized for extractable P. The objectives of this research were to evaluate manure P extraction and sample storage procedures and to characterize manure types for water-extractable P (WEP) and  $\text{NaHCO}_3$  P (BiEP). Manure P was extracted at dry matter-to-water extraction ratios of 0.5 g/200 mL, 2 g/200 mL, 2 g/20 mL, and 20 g/200 mL. Shaking times of 0.5 h, 1 h, or 2 h were evaluated along with filter paper types (Whatman No. 42, Whatman No. 40, and 0.45- $\mu\text{m}$ ). Single or sequential extractions and repeated extractions with water or  $\text{NaHCO}_3$  were also compared on various manure sources. Manure types were treated as replications in the analysis of variance to reduce the probability of making a Type I error in applying the results to diverse manure types. Dry matter-to-water extraction ratios more concentrated than 1 g/100 mL removed less P than extraction at 1 g/200 mL, which removed a similar percentage of total P (TP) as 0.5 g/200 mL ratio. A single extraction with a 1 g/200 mL or more dilute ratio with 1 h of shaking time was found to give a good estimate of extractable P. Extracted manure P was similar for three sequential extractions of 1 g/100 mL dilution ratio compared to one extraction with 1 g/300 mL. Filter paper type did not affect the amount of P extracted. Phosphorus extraction was more consistent with

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Address correspondence to Martha Mamo, Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE 68583, USA. E-mail: mmamo3@unl.edu

samples stored dry as compared to refrigerated or frozen conditions. Extractible P in swine manure, as a percentage of TP, was more than for other manure types.

**Keywords:** Dilution ratios, extractible P, filter paper, shaking time

## INTRODUCTION

Land application of readily extractible phosphorus (P) in either mineral fertilizer or manure, and high soil-test P levels, increase the potential for total P (TP) and dissolved reactive P (DRP) loss in runoff during the weeks following application (Sharpley et al. 1988; Sharpley, Daniel, and Edwards 1993; Kleinman et al. 2002; McDowell and Sharpley 2001). Manure application to soils results in temporary increases in DRP at the soil surface, from which it can be transported in runoff. Variation in DRP loss in runoff may be a function of manure type (Sharpley et al. 1988). Kleinman et al. (2002) found high correlation between water-extractible P (WEP) concentration of dairy, poultry, and swine manure applied to the surface of three soils and DRP loss in runoff. Sharpley and Moyer (2000) found WEP to be closely related to potentially leachable manure and compost P. The forms of P added to soil, therefore, directly affect P availability to runoff.

The relationships of different manure P extractants and DRP concentration in runoff have been studied (Self-Davis and Moore 2000; Sharpley and Moyer 2000). Dou et al. (2000) designated P extracted by water and  $\text{NaHCO}_3$  (BiEP) as readily soluble and P extracted by HCl or trichloroacetic acid as somewhat soluble. After evaluating multiple sequential extractions and different shaking times with water and with  $\text{NaHCO}_3$ , Dou et al. (2000) suggested a single extraction of manure P with 1 h of shaking time.

Kleinman et al. (2002) compared different dilutions, shaking times, and filter paper types. Shaking for 1 h provided the strongest regression coefficient for relating WEP to runoff DRP, but WEP increased with dilution. Higher WEP was found in extracts filtered through Whatman No. 1 than those filtered with a 0.45- $\mu\text{m}$  filter, but filtration method did not affect the relationship between WEP and runoff DRP. There is a need to better understand the variation in WEP of manures given its importance to the potential for DRP runoff following manure application. A fast and inexpensive procedure is needed to determine the solubility of manure P for routine manure testing. The objectives of this study were to refine the procedures for storage of manure samples and for extraction of readily soluble manure P and to determine the variation in readily soluble P with manure types.

## MATERIALS AND METHODS

Five experiments were conducted using four sets of manure samples. The study of the effect of dilution, shaking time, and filter papers focused on

beef feedlot manure. The studies addressing repeated extractions and the comparison of single versus multiple sequential extractions used samples of six manure types to ensure relevance of the results to diverse manure types. These samples as well as the previously mentioned beef manure samples were not dried but had been stored under refrigeration since collection. The study of manure storage time and conditions required freshly collected manure samples, and four manure types were used. The study of manure types used 45 samples representing eight manure types; these samples were submitted for routine analyses by producers over a period of 13 months and were stored with refrigeration for 2 to 15 months.

### Dilution, Shaking Time, and Filter Papers

Optimal dilution ratio and shaking time were determined using fresh manure samples of beef cattle collected from the ground in four feedlots. Water, ash, and P concentration of these samples varied from 90 to 330, 500 to 760, and 17 to 21 g kg<sup>-1</sup>, respectively. The ratios of dry equivalent manure extracted with distilled deionized water were 1 g/200 mL, 2 g/200 mL, 2 g/20 mL, and 20 g/200 mL (Self-Davis and Moore 2000; Sharpley and Moyer 2000). Soil–water mixtures at different ratios were shaken for 0.5 h, 1 h, or 2 h on an oscillating shaker at 150 cycles min<sup>-1</sup>. Water-extractable P was measured by the molybdate method (Murphy and Riley 1962) following filtration through Whatman No. 42 filter paper under vacuum suction. The four manure samples were treated as replications in the analysis of variance.

The beef cattle feedlot manure samples were also extracted for 1 h with distilled deionized water at dilution ratios of 0.5, 1, and 2 g dry equivalent manure to 200 mL to compare Whatman No. 42, Whatman No. 40, and 0.45- $\mu$ m filters. The four manure samples were treated as replications in the analysis of variance.

### Repeated Extractions

The effect of repeated extractions on WEP and BiEP was evaluated using samples of six manure types according to the method of Dou et al. (2000). The six manure samples were finishing swine solid from a concrete floor, finishing beef solid from a dirt feedlot, poultry solid from a pit, beef yearling solid from a concrete floor, finishing beef slurry from a pit, and finishing swine from a one-stage lagoon. Manure samples were extracted with water four times, each with 1 h of shaking, followed by three 30-mL 0.5 M NaHCO<sub>3</sub> extractions, each with 1 h of shaking. The sample mass to volume was 0.3 g dry equivalent manure to 30 mL extractant. After each extraction, the samples were filtered through Whatman No. 42 filter paper using vacuum filtration followed by P analysis with the molybdate method (Murphy and

Riley 1962). The cumulative percentage of TP extracted at each stage was determined. Manure samples were treated as replications in the analysis of variance.

### Single versus Multiple Sequential Extractions

The effect of single versus multiple extractions on WEP and BiEP was evaluated using the six manure samples used in the repeated extractions study. Phosphorus was sequentially extracted with distilled, deionized water followed by 0.5 M NaHCO<sub>3</sub> in a single- or multiple-extraction procedure. For the single extraction, 0.3 g of dry equivalent manure was extracted for 1 h with 90 mL of distilled deionized water followed by 1 h of shaking with 90 mL of 0.5 M NaHCO<sub>3</sub>. For the sequential extraction, 0.3 g of dry equivalent manure was extracted three times in sequence in 30 mL of distilled, deionized water followed by three extractions with 30 mL of 0.5 M NaHCO<sub>3</sub> with 1 h of shaking for each extraction. After each extraction, the samples were filtered through Whatman No. 42 filter paper using vacuum filtration. Samples collected on the filter paper were returned to the extracting flask by rinsing with a known volume of water or 0.5 M NaHCO<sub>3</sub>, depending on which extractant followed in the sequence. Additional extractant was added to the flask to bring the volume to 30 mL, and the extraction procedure was repeated. The significance of differences in P extracted with single versus multiple extractions was determined both for WEP and BiEP. Manure samples were treated as replications in the analysis of variance.

### Manure Storage Time and Conditions

The effect of storage time and conditions on WEP was determined using fresh samples of four manure types: swine slurry, beef feedlot manure, poultry manure, and composted feedlot manure. Manure WEP was measured after 0, 4, 7, 14, and 28 days of storage. The storage conditions included air-dry storage, refrigeration ( $4 \pm 2^\circ\text{C}$ ), and freezing ( $-20 \pm 2^\circ\text{C}$ ). At the end of each storage period, samples were shaken for 1 h at a ratio of 1 g of dry equivalent manure to 200 mL of distilled deionized water. Samples were filtered through Whatman No. 42 and analyzed for P by the molybdate method (Murphy and Riley 1962). Two samples of each manure type in each storage condition were analyzed for WEP after each storage period.

### Manure Type

Forty-five samples of dairy, beef, poultry, and swine manure from various manure storage and handling systems were evaluated to compare TP, WEP, and BiEP. One sample of 0.3 g of air-dry-weight equivalent for each of the

45 manure types was extracted with 90 mL of deionized distilled water to determine WEP, followed by a sequential extraction with 90 mL of 0.5 M  $\text{NaHCO}_3$ . Shaking time for each extraction was 1 h. Filtration and P determination were as for the dilution and shaking time study.

### Data Analyses

In all studies except the study of storage time and conditions, manure types were treated as replications in the analysis of variance, rather than using several replications of a single sample. This was done to reduce the probability of making a Type I error in applying the results to diverse manure types. In the manure-type study, the effects of manure type on TP, WEP, and BiEP were determined using a mixed model of SAS (Littell et al. 1999). Mean separation, at  $P < 10\%$ , were done using SAS version 8.0 (SAS Institute 1999). The sample storage study was conducted with four manure types and two replications.

## RESULTS AND DISCUSSION

### Dilution Ratios and Shaking Times for WEP

Manure WEP for the four manures ranged from 7.5 to 30.5% of TP (Table 1). The effects of extraction ratio, shaking time, and extraction ratio by shaking

**Table 1.** Effect of shaking time and dilution ratios (dry manure equivalent) on water-extractable P (WEP) [ $\text{mg kg}^{-1}$ , (% of TP)] (extractions were of fresh manure samples from four beef feedlots)

Ratios (g:mL)	0.5 h	1 h	2 h
1:200	1,470 (20.9) aA	1,991 (28.3) aB	2,157 (30.5) aB
2:200	1,391 (19.8) aA	1,666 (23.7) bB	1,816 (25.7) bB
2:20	542 (7.5) bA	586 (8.2) cA	678 (9.6) cA
20:200	542 (7.6) bA	586 (8.2) cA	651 (9.2) cA
ANOVA		$P > F$	
Time		0.0001	
Ratio		0.0001	
Time $\times$ ratio		0.0155	

*Notes.* Numbers in parentheses are WEP as percent of total manure P (%TP).

First letters compare means of ratios within a shaking time (column). Means with the same are not significantly different at 10% using ANOVA-protected LSD means comparison.

Second letters compare means of shaking time within a ratio (row). Means with the same are not significantly different at 10% using ANOVA-protected LSD means comparison.

time interaction were significant. The coefficients of variability as a measure of repeatability were lower for 1:200 and 2:200 than for 1:20 and 20:200. The 2:20 and 20:200 dilutions had similar WEP but less WEP than for the 1:200 and 2:200 dilutions. Dilution ratios of 1:200 and 2:200 extracted similar amounts of WEP as a percent of total manure P. Kleinman et al. (2002) also observed more P extraction with greater dilution and concluded that the increase in WEP to be associated with dissolution of insoluble Ca-P. Manure WEP was less with 0.5 h of shaking but was similar for 1 h and 2 h shaking time at the 1:200 and 2:200 ratios. This finding agrees with the results of Dou et al. (2000), where they observed increased WEP with increased shaking time, but they concluded that the observed increases were relatively small and recommended the use of 1 h of shaking time.

### Filter Papers

The percent of TP extracted as WEP ranged from 27.0 to 30.5% of total manure P (Table 2) with no effect of filter paper type. The interactions of filter paper with shaking time and with dilution ratio were not significant. Although dissolved reactive P has been considered to be P that passes through a 0.45- $\mu$ m filter (Dou et al. 2000), other filter papers can be used for WEP determination. Manure WEP, as percent of TP, extracted at 2:200 ratio, was consistently lower than the 1:200 and 0.5:200 ratios with all filter

**Table 2.** Effects of dilution ratios (dry manure equivalent to water) and filter paper on water-extractable P (WEP) [ $\text{g kg}^{-1}$  (% of TP)]

Ratios (g:mL)	Filter 42	Filter 40	0.45- $\mu$ m Filter
0.5:200	2.4 (34.0) aA	2.4 (33.6) aA	2.5 (35.1) aA
1:200	2.3 (32.4) aA	2.4 (33.1) aA	2.4 (33.4) aA
2:200	1.9 (27.2) bA	1.9 (27.0) bA	1.9 (27.1) bA
ANOVA	$P > F$		
Paper	0.9111		
Ratio	0.0001		
Paper $\times$ ratio	0.9960		

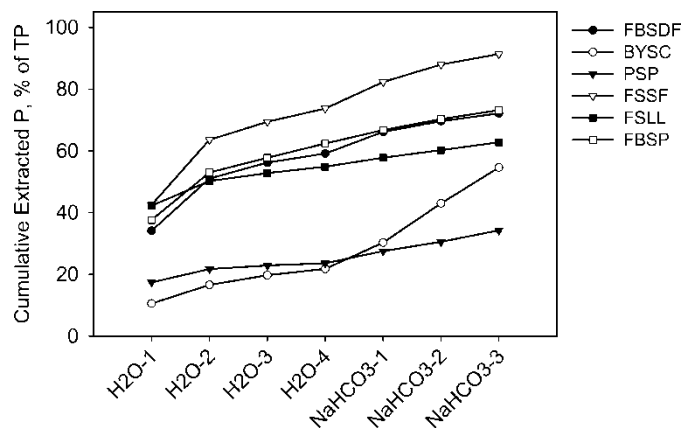
*Notes.* Extractions were of fresh beef manure samples from four feedlots shaken for 2 h. Number in parentheses are WEP as %TP.

First letters compare means of ratios within a filter type (column).

Means with the same letters are not significantly different at 10% using ANOVA-protected LSD means comparison.

Second letters compare means of filter paper within a ratio (row).

Means with the same letters are not significantly different at 10% using ANOVA-protected LSD means comparison.



**Figure 1.** Cumulative P extracted from various manures by sequential extraction with de-ionized distilled water followed by extraction with  $\text{NaHCO}_3$  and expressed as a percentage of TP. Each extraction was done at a dry matter-to-extractant ratio of  $0.3 \text{ g}/30 \text{ mL}^{-1}$  (1:100). P extracted was linearly related to number of extractions for all manure types ( $P > 0.01$ ;  $R^2 > 0.90$ ). FBSDf = solid manure of finishing beef on dirt feedlot; FBSP = pit slurry of finishing beef; BYSC = solid manure of beef yearlings on concrete; FSSF = solid manure of finishing swine on a concrete floor; FSLl = lagoon slurry of finishing swine; PSP = solid pit manure of poultry.

paper types. This second finding agrees with the results of the dilution ratio and shaking time experiment discussed previously.

### Repeated Extractions

Cumulative extracted P differed for manure types at each extraction stage (Figure 1). When using a 1:100 ratio of manure to distilled water or  $\text{NaHCO}_3$ , WEP and BiEP, as percentage of TP, continued to increase with added extractions (Figure 1). Linear increases in extracted P were significant for all manure types. The average increase in extractable P for manure types differed, ranging from 2.6 to 7.0% of TP for each extraction for poultry and yearling beef manure, respectively. Mean WEP was 54% more with four extractions as compared to the first extraction. Mean BiEP was 21% more following three as compared to the first  $\text{NaHCO}_3$  extraction. The patterns of increasing WEP and BiEP with more extractions were comparable to those reported by Dou et al. (2000).

### Single versus Multiple Sequential Extractions

Amounts of WEP and BiEP extracted with a single 1:300 dilution ratio versus three extractions with 1:100 ratio were not significantly different

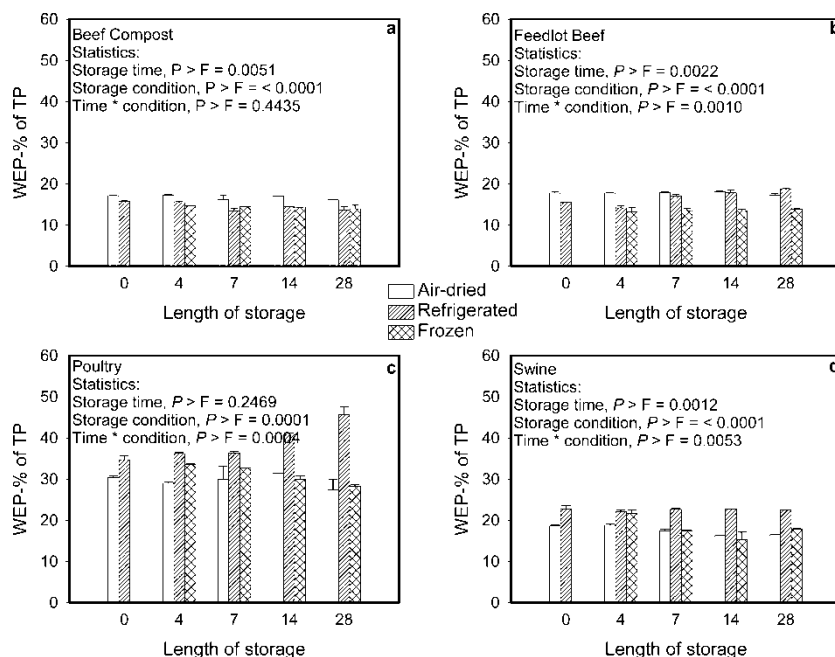


**Table 3.** Single extraction with a 1:300 dry manure equivalent-to-water dilution ratio compared to three extractions with a 1:100 ratio for water (WEP) and NaHCO<sub>3</sub> (BiEP) extractable P

Source	%DM	Total P (g kg <sup>-1</sup> )	WEP (1 extraction) (g kg <sup>-1</sup> )	WEP (3 extractions) (g kg <sup>-1</sup> )	BiEP (1 extraction) (g kg <sup>-1</sup> )	BiEP (3 extractions) (g kg <sup>-1</sup> )
FBSDF	78	5.5	3.5	3.1	0.61	0.71
BYSC	21	5.7	1.1	1.1	1.8	2.1
PSP	42	19.4	4.1	3.9	1.6	1.8
FSSF	31	19.6	12.2	13.6	4.5	3.5
FSL	4	26.6	13.3	13.8	2.9	2.1
FBSP	13	12.2	5.0	7.1	2.4	1.3
Significance <sup>a</sup>			ns		ns	

*Notes.* FBSDF = solid manure of finishing beef on dirt feedlot; BYSC = solid manure of beef yearlings on concrete; PSP = solid pit manure of poultry; FSSF = solid manure of finishing swine on a concrete floor; FSL = lagoon slurry of finishing swine; FBSP = pit slurry of finishing beef.

<sup>a</sup>Compares effects of number of extractions.



**Figure 2.** Effect of the interaction of manure type, sample storage conditions, and storage time on percentage of TP that is water extractable.

(Table 3). These results are in agreement with Dou et al. (2000), who concluded that a single extraction with a lower manure-to-water ratio was adequate for reliable results.

### Manure Storage Time and Conditions

The main effects and all two- and three-way interaction effects (i.e., manure type by storage time by storage condition) were significant, except for the main effect of storage time. The interaction of manure type by storage conditions was primarily due to increase in poultry and swine manure WEP with refrigeration as compared to other storage conditions (Figure 2). This increase suggests that even at low refrigeration temperature, P hydrolysis occurs. The three-way interaction of manure type by storage condition by storage time was due to the increase with time of poultry manure WEP with refrigeration. Manure WEP was most stable across storage conditions and storage time for compost and feedlot manures and least stable for poultry manure. WEP was most stable with dried manure and least stable with refrigeration. Refrigeration of samples is recommended if storage is less than 7 days because even at 2 to 4°C there is slow microbial activity

(Coxson and Parkinson 1987) Dried manure was stable but samples should be air-dried rather than oven-dried as P transformations can occur when oven-drying (Ajiboye, Akinremi, and Racz 2004).

### WEP and BiEP of Different Manure Types

The eight sets of animal-manure types differed widely in water and nutrient content (Table 4). Total P was highest for swine manure, followed by poultry and beef manure. Total P in beef cattle manures did not differ for species age, but liquid manure contained more P than solid manures on a dry-weight basis (Table 5). Beef manure from dirt feedlots contained soil as indicated by manure ash content, which was related to TP content ( $r^2 = 0.47$ ,  $P < 0.1$ ) but not to extractable P. Swine manure from lagoon storage had a higher P concentration on a dry-weight basis than other swine manures.

Manure TP, WEP, and often BiEP, based on the dry weight of manure ( $\text{g kg}^{-1}$ ), were higher for manure of finishing swine than of other animal groups and were highest with lagoon slurry of finishing swine (Table 5). Water-extractable P as a percent of TP was least with poultry manure (21%) and most with solid swine manure (58%), suggesting that most of the TP in this swine manure is in highly soluble P forms. Additional P extracted by  $\text{NaHCO}_3$ , as a percentage of TP, was generally more for beef than swine or poultry, suggesting presence of more recalcitrant P in swine and poultry (Table 5).

Assuming that WEP is the best indicator of the potential for contamination of surface water with dissolved reactive P, the greatest concern is

**Table 4.** Mean values for eight animal-manure types consisting of a total of 45 samples, on a fresh weight basis, evaluated for extractable P

Manure type	N	DM (%)	Organic N ( $\text{mg kg}^{-1}$ )	$\text{NH}_4$ ( $\text{mg kg}^{-1}$ )	Ca ( $\text{mg kg}^{-1}$ )	EC ( $\text{dS m}^{-1}$ )
FBSDF	15	64.4	9,673	2,706	11,962	2.4
FBLL	3	0.8	219	415	364	0.79
FBSP	3	13.2	2,228	4,314	2,772	3.1
BYSC	3	18.3	23,733	2,769	19,500	3.7
FSSF	12	27.2	37,833	7,111	22,408	3.3
FSLI	3	2.6	666	1,993	1,247	1.4
FaSLI	3	1.5	492	1,296	763	1.1
PSP	3	36.3	15,033	20,271	145,100	6.2

*Notes.* FBSDF = solid manure of finishing beef on dirt feedlot; FBLL = lagoon liquid of finishing beef; FBSP = pit slurry of finishing beef; BYSC = solid manure of beef yearlings on concrete; FSSF = solid manure of finishing swine on a concrete floor; FSLI = lagoon slurry of finishing swine; FaSLI = lagoon slurry of farrowing swine; PSP = solid pit manure of poultry.

**Table 5.** Total P (TP) on a dry weight basis, and water (WEP) and NaHCO<sub>3</sub> extractible P (BiEP) expressed as dry-weight concentrations and as percent of TP in parenthesis, of 45 manure samples in eight animal-manure types

Manure type	N	TP		WEP		BiEP	
		g kg <sup>-1</sup>	CV%	g kg <sup>-1</sup> (%TP)	CV%	g kg <sup>-1</sup> (%TP)	CV%
FBSDF	15	3.4 c	32	1.6 de (43.8)	54	0.8 d (24.2)	35
FBLL	3	12.3 bc	30	3.8 cde (30.5)	50	5.2 ab (44.1)	18
FBSP	3	12.7 bc	9	5.9 c (46.0)	20	1.8 cd (14.4)	31
BYSC	3	3.8 c	43	0.8 e (21.9)	32	0.9 d (21.7)	85
FSSF	12	18.4 b	23	10.0 b (57.8)	18	3.8 bc (19.5)	50
FSLI	3	46.6 a	53	16.7 a (38.9)	34	6.5 a (13.6)	57
FaSLI	3	56.2 a	34	18.0 a (34.7)	25	6.5 a (12.3)	9
PSP	3	23.4 b	23	4.7 cd (20.6)	10	2.4 cd (10.0)	29

*Notes.* The single extraction with water was followed by an extraction with NaHCO<sub>3</sub>, each at 1:100 (0.3 g/30 mL) dilution ratio.

FBSDF = solid manure of finishing beef on dirt feedlot; FBLL = lagoon liquid of finishing beef; FBSP = pit slurry of finishing beef; BYSC = solid manure of beef yearlings on concrete; FSSF = solid manure of finishing swine on a concrete floor; FSLI = lagoon slurry of finishing swine; FaSLI = lagoon slurry of farrowing swine; PSP = solid pit manure of poultry.

Means with the same letters across columns are not significantly different at 10% using ANOVA-protected LSD means comparison.

with swine and beef manure and the least concern with poultry manure when similar rates of TP are land applied. The validity of this assumption, however, is dependent on water content of the applied manure as some WEP moves a short distance into the soil with liquid manures, where it is less exposed to runoff loss. Phosphorus availability to plants in the first season after application, for a given P application rate, is expected to be greater for finishing swine and beef manure and less for manure of poultry, farrowing swine, or yearling beef.

## CONCLUSIONS

Extraction ratio and shaking time significantly affected WEP. WEP can be well estimated with a dilution ratio of 1:200 and a shaking time of 1 h. For routine manure analyses, the less expensive filters such as Whatman Nos. 42 and 40 can be used instead of 0.45- $\mu\text{m}$  filter discs. For routine manure analyses, samples are better stored air dried rather than refrigerated or frozen. There was relatively more TP as WEP in solid swine and finishing beef manures compared to other manures. More of TP in swine and finishing beef manures is likely to be plant available but with more risk of dissolved runoff P loss shortly after manure is applied.

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